Epoxidation of Polyunsaturated Fatty Acid Double Bonds by Dioxirane Reagent: Regioselectivity and Lipid Supramolecular Organization

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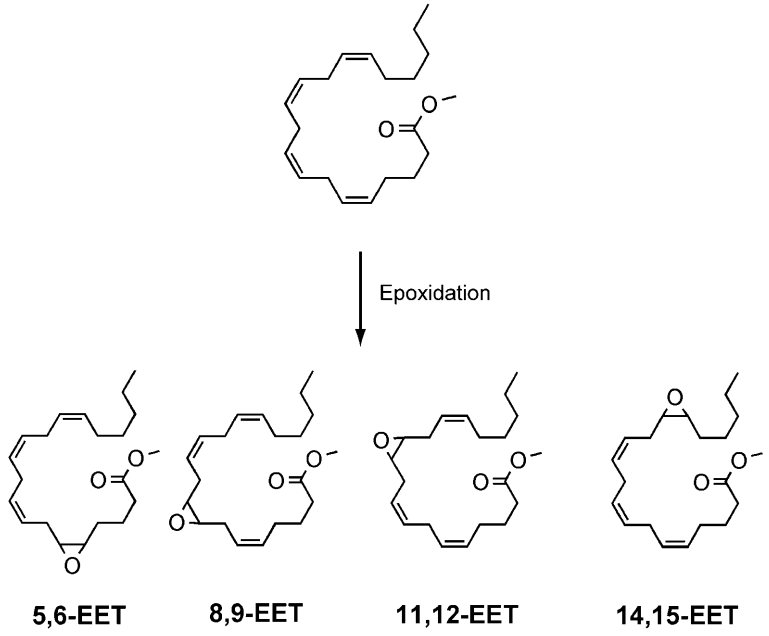
Dedicated to Professor *Hanns Fischer*, an unforgettable teacher and friend

The use of dimethyldioxirane (DMD) as the epoxidizing agent for polyunsaturated fatty acids was investigated. With fatty acid methyl esters, this is a convenient method for avoiding acidic conditions, using different solvents, and simplifying the isolation procedures, with less contamination due to by-products. The reagent was also tested with free fatty acids in water. In this case, the supramolecular organization of fatty acids influenced the reaction outcome, and the epoxidation showed interesting regioselective features. The C=C bonds closest to the aqueous-micelle interface is the most favored for the interaction with dimethyldioxirane. The preferential epoxidation of linoleic acid (=(9*Z*,12*Z*)-octadeca-9,12-dienoic acid) to the 9,10-monoepoxy derivative was achieved, with a high yield and 65% regioselectivity. In case of arachidonic acid (=(5*Z*,8*Z*,11*Z*,14*Z*)-eicosa-5,8,11,14-tetraenoic acid) micelles, the regioselective outcome with formation of the four possible monoepoxy isomers was studied under different conditions. It resulted to be a convenient synthesis of '*cis*-5,6-epoxyeicosatrienoic acid' (=3-[(2*Z*,5*Z*,8*Z*)-tetradeca-2,5,8-trienyl]oxiran-2-butanoic acid), whereas in reverse micelles, epoxidation mostly gave '*cis*-14,15-epoxyeicosatrienoic acid (=(5*Z*,8*Z*,11*Z*)-13-(3-pentyloxiran-2-yl)trideca-5,8,11-trienoic acid).

Introduction. – After the early discovery as constituents of vegetable oils [1] and metabolites by the epoxygenase pathway [2], epoxy derivatives of polyunsaturated fatty acid (PUFA) have attracted a multidisciplinary research interest in chemistry, biology and pharmacology. In particular, monoepoxy derivatives of linoleic acid ((9*Z*,12*Z*)-octadeca-9,12-dienoic acid) and arachidonic acid (=(5*Z*,8*Z*,11*Z*,14*Z*)-eicosa-5,8,11,14-tetraenoic acid) have been identified as products of cytochrome P450 enzymes, and a series of biological effects have been linked to their *in vivo* formation. '*cis*-9,10-Epoxy-12*cis*-octadec-12-enoic acid' (=*rel*-(2*R*,3*S*)-3-[(2*Z*)-oct-2-enyl]oxiraneoctanoic acid; 9,10-EOA) and '*cis*-12,13-epoxy-9*cis*-octadecenoic acid' (=*rel*-(9*Z*)-11-[(2*R*,3*S*)-3-pentyloxiran-2-yl]undec-9-enoic acid; 12,13-EOA) are known as leukotoxin and isoleukotoxin, respectively, and are formed during the functioning of leukocytes, as well as the lipid peroxidation processes [3]. In the field of natural-product chemistry, 9,10-EOA and 12,13-EOA are known as coronaric and vernolic acids, respectively [1]. The '*cis*-epoxyeicosatrienoic acids' (*cis*-EETs, *Scheme 1*) are synthesized in many tissues, including kidney, liver, adrenal cells, ovarian cells, endothelial cells and brain. Potent effects have been observed in modulating various ion channels, membrane-bound transport proteins, mitogenesis, tyrosine kinase cascades [4]. Specific

Scheme 1. *Preparation of Four Regioisomers by Monoepoxidation of Arachidonic Acid Methyl Ester*



activities have been linked to each regioisomer of PUFA monoepoxides, as for example, the previously recalled leukotoxin which is specifically produced and hydrolyzed to the corresponding diol in the acute respiratory distress syndrome [5], or the 8,9- and 11,12-EET, which are activators of K_{ATP} channels with a resulting modulation of cardiac electrophysiology [6]. In case of the 14,15-EET, this compound is known as an epidermal growth-factor signaling [7].

Therefore, fatty acid epoxidation addressing the regioselectivity issue is of relevant importance. Chemical methods are limited to the classical procedure with peroxy acid reagents, such as 3-chloroperbenzoic acid (*m*-CPBA), which are essentially nonselective toward the different C=C bonds of polyunsaturated structures. Unselective bis-epoxidation of linoleic acid and with *m*-CPBA, has been used in the route to tetrahydrofurans [8]. In the case of arachidonic acid, the four monoepoxy derivatives are obtained (*Scheme 1*). The only selective epoxidation protocol to date is the 'internal' strategy reported by *Corey* and co-workers [9] applied to peroxyarachidonic acid, in which an intramolecular O-transfer operates to afford preferentially the 14,15-EET. However, the peroxy acid method does not avoid acidic conditions and the need of chromatographic removal of by-products. For conjugated linoleic acid, a recent report comparing different epoxidizing conditions has been published, without evidencing any selectivity feature [10].

In connection with our work on the regioselectivity of radical reactions involving the supramolecular organization of a lipid assembly [11][12], the study of the epoxidation with dimethyldioxirane (DMD) [13][14] can be interesting, based on two features: *i*) the advantage of nonacidic reaction conditions, coupled with the production of vol-

atile by-products and the easy isolation procedure; *ii*) the tolerance of DMD to aqueous conditions. This latter aspect can be crucial for a regioselective strategy. In fact, fatty acids are immiscible in water and assume a supramolecular organization, forming micelles at a critical micelle concentration (CMC). The supramolecular disposition of the hydrophobic fatty acid chains in this assembly can drive specific interactions with the added reagents, so that molecular properties can be developed at the interface between H_2O and hydrophobic phases. This concept has been recently applied in several aqueous synthetic procedures 'on water', the immiscibility of the reagents becoming an advantage instead of being a drawback [15][16]. The behavior in micelles can also be compared with reverse micelles that fatty acids form in organic solvents, with an organization opposite to the aqueous conditions.

We now report the results of epoxidation of linoleic and arachidonic acid and their methyl esters with DMD, together with the study of regioselectivity obtained with the free acids in micelles.

Results. – 1. *PUFA Methyl Ester Epoxidation.* The 0.08–1.4M solution of DMD in acetone was synthesized following the standard procedure from *Oxone*® (2 $KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) [13][14]. To perform the epoxidation in other organic solvents, an extraction from acetone was preliminarily carried out to get a final DMD concentration of 0.15M, as determined by spectrophotometric measurement [14]. First, we tested the reactivity of linoleic acid methyl ester with an equimolar amount of DMD under different conditions of solvent and temperature. The results are reported in *Table 1*. As depicted in *Scheme 2*, the monoepoxy derivatives, 9,10-EOA (**1**) and 12,13-EOA (**2**) are the intermediates for the half reaction.

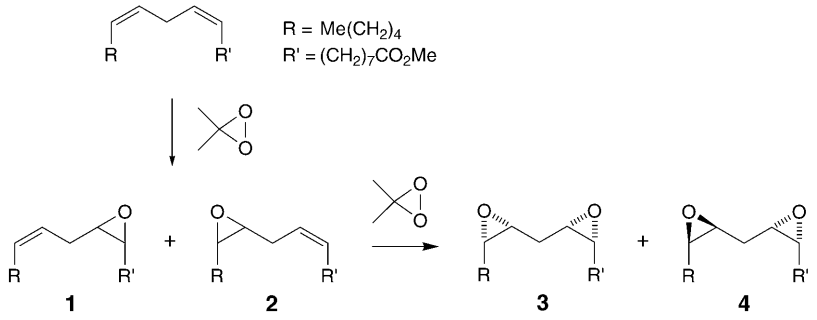
Table 1. *DMD Epoxidation of Linoleic Acid Methyl Ester* (see *Scheme 2*)

Entry	Temperature [°], solvent, equiv. DMD	Yield [%]	Monoepoxy derivatives **1/2**	Diepoxy derivatives **3/4**
1	0, CH_2Cl_2, 1	51[a])	1:1 (47%)	1:3 (4%)
2	−20, CH_2Cl_2, 1	50[a])	–	1:2.3
3	0, CH_2Cl_2, 2	99	–	1:2.3
4	0, acetone, 2	99	–	1:1.7
5	45, acetone, 2	99	–	1:1.7

[a]) 49% recovery of starting material.

The best solvent and temperature for monoepoxidation were found to be CH_2Cl_2 at 0° and 1 equiv. of DMD (*Table 1*, *Entry 1*). Under these conditions, only a 4% yield of diepoxy derivatives was obtained, whereas the two positional isomers were equally formed in 47% yield, and a full recovery of the starting methyl ester was possible. The same reaction at −20° (*Entry 2*) afforded only '*syn*' and '*anti*' diepoxy derivatives **3** and **4** in 50% yield. With 2 equiv. of DMD and raising the temperature to 0° to improve the solubility, **3** and **4** were obtained in quantitative yield (*Entry 3*). By replacing CH_2Cl_2 with acetone as the solvent, a similar result was obtained (*Entry 4*), which did not change at higher temperature (*Entry 5*). The two solvents slightly influenced the '*syn*' and '*anti*' diepoxy derivative ratio, which is always in favor of '*anti*' **4** and similar to

Scheme 2. *Synthesis of Mono- and Diepoxy Derivatives by DMD Epoxidation of Methyl Linoleate*



the reaction with peracids [17]. The separation between the '*syn*' and '*anti*' products was achieved by crystallization at $-20°$ in hexane, and their spectroscopic characteristics were in agreement with the literature data [18].

The methodology was then applied to methyl arachidonate. *Fig. 1* shows the formation of the epoxy products, and each point corresponds to the experiment run with the corresponding amount of DMD. For example, a *ca.* 40% yield of monoepoxy derivatives was obtained with 0.5 equiv. DMD, which also afforded 4% of diepoxy derivatives. Based the recovery of the unreacted starting material, the yield was quantitative. As shown in *Fig. 1*, formation of di- and triepoxy derivatives increased with higher amounts of DMD.

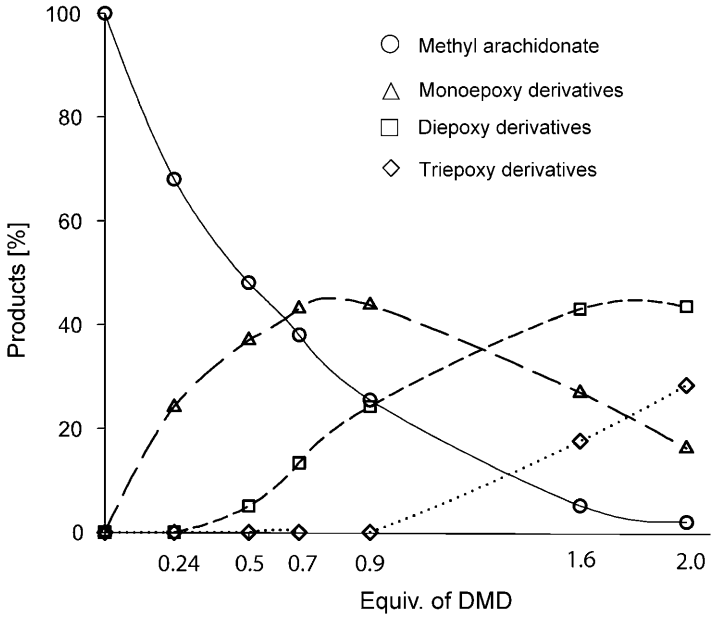


Fig. 1. *Product distribution in the epoxidation of methyl arachidonate with DMD.* The equiv. of DMD refer to 1 equiv. of substrate, in CCl_4 at 0°.

Silica gel chromatography is a well known method for the separation of EET as methyl esters [9]. The isolated monoepoxy derivatives were identified by comparison with commercially available references, resulting in a mixture of 5,6-EET/8,9-EET/11,12-EET/14,15-EET in the ratio of 1.6 : 1.6 : 2.6 : 1.

The exhaustive epoxidation of methyl arachidonate was carried out with 4 equiv. of DMD in CCl_4 at 0° with a quantitative formation of a mixture of the diastereoisomeric tetraepoxy derivatives. The presence of diastereoisomeric tetraepoxy derivatives was also confirmed by GC and GC/MS analyses, but the mixture was not further resolved.

2. *PUFA Epoxidation in Micelle.* The epoxidation was then performed under aqueous conditions with free fatty acids, which is very convenient for a direct access to the biologically active compounds. In H_2O the CMCs for linoleic and arachidonic acids are *ca.* 10^{-4}M [19], and the supramolecular arrangement of the fatty acid chains in the micelles could be relevant for the regioselectivity of the transformation. An optimization of the fatty acid concentrations and pH conditions was preliminarily carried out. DMD in H_2O was formed by an *in situ* procedure, *i.e.*, by adding the potassium peroxymonosulfate salt to a vigorously stirred basic suspension of the fatty acid, containing $<2\%$ of acetone [20]. In case of linoleic acid, a 0.1M fatty acid suspension was prepared in aqueous 0.9M KOH containing 1.12M $NaHCO_3$ (pH 9.5), and *Oxone*® was added portion-wise up to a final concentration of 0.05M. The mixture was then stirred overnight at room temperature, followed by extraction and transformation of the fatty acids to the corresponding methyl esters by diazomethane for GC analyses. Under these conditions, a 15% conversion was found with a complete recovery of the starting linoleic acid (85%). Interestingly, the resulting mixture of monoepoxy derivatives was in favor of the 9,10-EOA (**1**), the 9,10-EOA/12,13-EOA (**1**/**2**) ratio being 1.8 : 1. Remarkably, this is the first report of regioselective epoxidation of linoleic acid.

The more complex case of arachidonic acid epoxidation in a micelle environment was then examined. A vigorously stirred 0.05M suspension of arachidonic acid was used in the above described alkaline aqueous medium. DMD was progressively formed *via* the *in situ* addition (in portions) of *Oxone*® during a few hours. The progress of the reaction was monitored by TLC (silica gel), which allows arachidonic acid to be separated from the monoepoxy, and also from the disepoxy derivatives. On TLC, the monoepoxy regioisomer 14,15-EET (R_f 0.62) was separated from 11,12-EET/8,9-EET (R_f 0.58) and 5,6-EET (R_f 0.50). The results of the product distribution at two different *Oxone*® concentrations are shown in *Table 2* (*Entries 1* and *2*).

In the first case, a total of 1.9 equiv. of *Oxone*® was added (*Table 2*, *Entry 1*). Due to the mild conditions, easy workup, and good analytical procedures, a quantitative material balance was obtained, composed by 46% of monoepoxy derivatives, 27% of diepoxy derivatives, and 27% of recovered starting material. The identification of the EETs was performed by isolation of the regioisomers by means of silica gel chromatography (see above), characterization by NMR spectroscopy, and comparison with authentic references which are all commercially available. The final mixture of monoepoxy derivatives consisted of equal amounts of 5,6- and 8,9-EETs, and *ca.* 3–4 times less amount of 11,12-EET and 14,15-EET. This indicated that in micelles the C=C bonds have a nonequivalent reactivity toward the epoxidizing agent, with the C(5)=C(6) and C(8)=C(9) bonds more reactive compared to the other two positions. It is worth noting that in this reaction the 5,6-EET was isolated as a pure product in 15% yield.

Table 2. *DMD Epoxidation of Arachidonic Acid* (AA) *in Organized Systems*

Entry	AA [M]	Solvent, micelle type, reagent	Monoepoxy derivatives (yield [%])	5,6-EET/8,9-EET/ 11,12-EET/14,15-EET	Diepoxy derivatives (yield [%])	Recovered AA [%]
1	0.19	H_2O, micelles, 1.9 equiv. *Oxone*®	46[a])	3 : 3 : 1 : 0.7	27	27
2	0.19	H_2O, micelles, 0.8 equiv. *Oxone*®	30	3 : 1 : 0.3 : 0	15	55
3	0.035	CCl_4, reverse micelles, 0.8 equiv. DMD	47	1 : 1.3 : 3.3 : 4.5	19	23
4	0.030	CCl_4, reverse micelles, 1.9 equiv. DMD	23	0 : 1 : 3 : 3	44[b])	2

[a]) 5,6-EET in a 15% isolated yield. [b]) Formation of triepoxy derivatives (24% yield).

With 0.8 equiv. of *Oxone*®, a lower conversion was observed, *i.e.*, 30% of monoepoxy derivatives, 15% of diepoxy derivatives, and complete recovery of the unreacted arachidonic acid (55%) (*Table 2*, *Entry 2*). Interestingly, in this low-conversion protocol, a better regioselectivity was observed, the mixture of monoepoxy derivatives being highly in favor of the 5,6-EET regioisomer, present in a 3 : 1 : 0.3 ratio with 8,9-EET and 11,12-EET, whereas the 14,15-EET was completely absent.

Finally, arachidonic acid epoxidation was also run in CCl_4 as the solvent where a reverse micelle organization can be expected [21]. In this assembly, a different disposition of the hydrophobic chains occurs. *Table 2* shows the results for low- and high-conversion protocols (*Entries 3* and *4*), which were carried out by the direct DMD addition to the reaction mixture. It is worth noting that in the direct addition, a better control of the DMD is achieved, and the efficiency of the reaction in the organic solvent was much higher than in the aqueous system. The arachidonic acid concentration was optimized at *ca.* 0.030–0.035M. DMD was first added in a 0.8 equiv. amount with respect to the starting material, and the reaction was instantaneous (*Table 2*, *Entry 3*). After a few minutes, the workup and transformation to methyl esters were performed, resulting in 47% of monoepoxy and 19% of diepoxy derivatives. After silica gel chromatography, the monoepoxy fraction had the composition 14,15-EET/11,12-EET/8,9-EET/5,6-EET 4.5 : 3.3 : 1.3 : 1. This is in agreement with the supramolecular organization in the micelle system that differentiates the reactivity of the C=C bonds. Interestingly, the regioisomer composition was different from that obtained in the previously reported micelle experiments.

The experiment was repeated with 1.9 equiv. of DMD, but this time, the mixture of the epoxy derivatives was much more complicated since 24% of triepoxy derivatives were also present (*Table 2*, *Entry 4*), besides 23% of monoepoxy and 44% of diepoxy derivatives. Only a 2% of starting material was recovered. The EET mixture was composed of 14,15-EET/11,12-EET/8,9-EET in a 3 : 3 : 1 ratio.

Discussion. – PUFA Epoxidation with DMD proved to be convenient for avoiding acidic conditions and facilitating workup and isolation of the products. The epoxidation of linoleic acid methyl ester with DMD furnished the same results as the classical peracid method both for mono- and diepoxy derivatives [17]. The epoxidation of methyl arachidonate with DMD in CCl_4 is a step-by-step process, as depicted in *Fig. 1*. With 0.5 equiv. of DMD, the four monoepoxy derivatives 5,6-EET/8,9-EET/11,12-EET/14,15-EET were obtained in the ratio 1.6 : 1.6 : 2.6 : 1 as the main products in a 40% of conversion. For comparison, the reported ratios obtained after *m*CPBA [22] and enzymatic [23] epoxidations are 1 : 2 : 2 : 2 and 1.5 : 1 : 1.6 : 5, respectively.

When a supramolecular organization of the fatty acid is involved, the regioselectivity of the reaction can improve. DMD Epoxidation of linoleic acid in micelles represents the first case of a regioselective process, with the preferential formation of the 9,10-monoepoxy derivative. It indicates how effective the micellar supramolecular arrangement can be in orienting the reactivity of the epoxidation toward the C=C bond closest to the aqueous phase. In fact, DMD is generated *in situ* by the addition of the potassium peroxymonosulfate salt to the suspension. Once formed, it diffuses from the aqueous phase to the micelle compartment and reacts with the C=C bonds. The C=C bonds in the micelle arrangement of polyunsaturated fatty acids result to be nonequivalent, C(9)=C(10) being closer to the aqueous phase than C(12)=C(13), as shown in *Fig. 2*. Therefore, the regioselective outcome is due to a combination of DMD diffusion, local concentration and C=C bond interaction allowed by the supramolecular assembly [24].

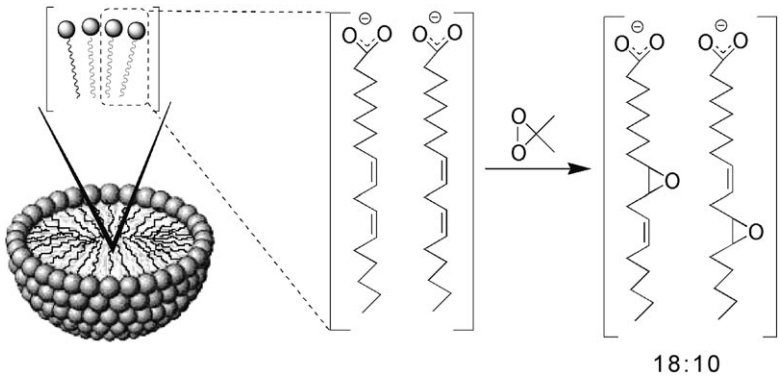


Fig. 2. *Sketch of linoleic acid micelles, and result of DMD epoxidation*

The regioselectivity is again found in the case of arachidonic acid epoxidation in micelles, where the preferential formation of 5,6-EET and 8,9-EET was observed. At lower conversion, that is, with less DMD equivalents, 5,6-EET is the prevalent isomer. In the absence of data on the most favored conformation of arachidonic acid in micelles, we can only say that the interaction of the epoxidizing agent with the first C=C bond of the chain occurs by a combination of factors, among which the diffusion of DMD and its local concentration play a role. At a low DMD concentration, chemoselectivity is achieved as well, with reduced formation of the diepoxy derivatives (*cf.*,

Table 2, *Entries 1* and *2*). Due to the satisfactory chromatographic separation of 5,6-EET, the DMD epoxidation in micelles can represent a convenient synthetic method for this biologically relevant monoepoxy derivative.

In CCl_4 the organization of free fatty acids takes the form of reverse micelles, and our data establish that in this case, 14,15-EET is the major compound. However, by using a higher DMD concentration, the selectivity drops, due to the local concentration that the reagent reaches in the organic-micellar interface, with a reduced yield of monoepoxy derivatives and increased formation of diepoxy derivatives. Under these conditions, 5,6-EET is not formed, which again can be explained by an effect of the local concentration of the reagent (*Table 2*, *Entry 4*).

Conclusions. – In this paper, a valuable strategy for the DMD epoxidation of fatty acids is reported, which expands the available selective synthetic methodologies for lipidomic libraries. Further work on the synthesis of specific mono-*trans* isomers of arachidonic acid [25], by using the micelle reactivity and formation of EET regioisomers, is in progress.

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Experimental Part

1. *General.* Arachidonic and linoleic acids and their corresponding methyl esters were commercially available from *Sigma-Aldrich*. EETs of arachidonic acid methyl ester were commercially available from *Biomol International* (Exeter, UK). The solns. of DMD (0.08–0.15M in CCl_4, ε_{max} 14 M^{-1} cm^{-1}) were prepared as already reported in detail [13][14]. HPLC-Grade CCl_4 and acetone were used as received. GC: *HP-5890-II* (gas chromatography; 30 m × 0.25 mm × 0.25 μm cross-linked 5% phenylsilicone capillary column (*HP-5*) with hexadecanoic acid methyl ester as the internal standard; initial temp. 100°, held for 2 min, then 20°/min up to 210°, held for 25 min. GC (linoleic acid reaction): *Varian-CP-3800* gas chromatograph; flame ionization detector, *Rtx-2330* column (90% biscyanopropyl/10% phenylcyanopropyl polysiloxane capillary column; 60 m, 0.25 mm i. d., 0.20 μm film thickness); initial temp. 160°, held for 55 min, then 5°/min up to 195°, held for 10 min, followed by a second increase of 10°/min up to 250° (FC = flash chromatography). UV-VIS Spectra: *Perkin-Elmer lamda-20* UV/VIS spectrometer with a thermostating block *Perkin-Elmer PTP-6* (*Peltier* system), in CCl_4. IR Spectra: *Perkin-Elmer Spectrum-BX-FT-IR* instrument; film or $CHCl_3$; in cm^{-1}. NMR Spectra: *Varian-Mercury* instrument at 400 (^1H) or 100.6 (^{13}C) MHz; δ in ppm; $CDCl_3$ as the solvent and the reference peak at δ 7.26. GC/MS: *HP-5890-II* instrument with a mass-selective detector *HP5972*. ESI/MS: *Bruker Esquire-3000-Plus* instrument; in *m*/*z*.

2. *Epoxidation of Linoleic Acid Methyl Ester.* 'cis-*9,10-Epoxy-12*cis-*octadecenoic Acid Methyl Ester*' (= rel-*(2*R,*3*S*)-3-[(2*Z*)-Oct-2-enyl]oxiraneoctanoic Acid Methyl Ester*; **1**) *and* 'cis-*12,13-Epoxy-9*cis-*octadecenoic Acid Methyl Ester* (= rel-*(9*Z*)-11-[(2*R,*3*S*)-3-Pentyloxiranyl]undec-9-enoic Acid Methyl Ester*; **2**). At 0°, 0.15M DMD in CCl_4 (2.2 ml, 0.34 mmol) was added to linoleic acid methyl ester (100 mg, 0.34 mmol) in CCl_4 (200 mL). The reaction of DMD was followed spectrophotometrically at 335 nm and monitored by TLC (Et_2O/hexane 1:4; R_f (starting material) 0.75, R_f (**1**) 0.49, R_f (**2**) 0.44, R_f (diepoxy derivative) 0.15). After a few minutes, the mixture was poured into brine and extracted with Et_2O. The

combined org. layer was dried ($MgSO_4$) and concentrated. The crude residue was purified by FC (25% Et_2O/hexane): recovered linoleic acid methyl ester (51 mg, 50%), **1** (24 mg, 22%), **2** (24 mg, 22%), having characteristics similar to those reported in [21].

'syn-' *and* 'anti-*9,10:12,13-Diepoxyoctadecanoic Acid Methyl Esters*' (= rel-*(2*R*,3*S*)-3-{[(2*R*,3*S*)-3-Pentyloxiran-2-yl]methyl}oxiraneoctanoic Acid Methyl Ester and* rel-*(2*R*,3*S*)-3-{[(2*S*,3*R*)-3-Pentyloxiran-2-yl]methyl}oxiraneoctanoic Acid Methyl Ester*, resp.; **3** and **4**, resp.). At 0°, 0.15M DMD in CCl_4 (0.07 mmol) was added to linoleic acid methyl ester (10 mg, 0.034 mmol) in CCl_4 (2 ml). The reaction of DMD proceeded as described above. After completion, the solvent was evaporated: **3**/**4** (11 mg, 99%). Separation of the diastereoisomers **3** and **4** was carried out according to the literature procedure. Spectroscopic data: identical to those reported in [8][17].

3. *Epoxidation of Arachidonic Acid Methyl Ester. Monoepoxy Derivatives.* At 0°, 0.15M DMD in CCl_4 (1 ml, 0.16 mmol) was added to arachidonic acid methyl ester (100 mg, 0.31 mmol) in CCl_4 (20 ml). The reaction of DMD was followed spectrophotometrically at 335 nm and was monitored by TLC (hexane/Et_2O 4:1) as described in [20]. The formation of monoepoxy derivatives was almost instantaneous, and the solvent was evaporated under vacuum. Chromatographic separation gave fractions of monoepoxy derivatives (42 mg, 40%), diepoxy derivatives (4 mg, 4%), and recovered starting material (54 mg, 55%). The monoepoxy derivatives were examined by ^{13}C-NMR: characteristic signals of epoxy and olefinic C-atoms of 5,6-EET/8,9-EET/11,12-EET/14,15-EET, the ratio being 1.6:1.6:2.6:1. Spectroscopic data (including MS): identical to references and in agreement with those reported in [9][22][23].

5,6:8,9:11,12:14,15-Tetraepoxyeicosanoic Acid Methyl Ester (=*3-{[3-{[3-[(3-Pentyloxiran-2-yl)methyl]oxiran-2-yl}methyl}oxiran-2-yl]methyl}oxiranebutanoic Acid Methyl Ester*). At 0°, 0.15M DMD in CCl_4 (0.9 ml, 0.13 mmol) was added to arachidonic acid methyl ester (10 mg, 0.033 mmol) in CCl_4 (2 ml). The reaction was followed spectrophotometrically at 335 nm and monitored by TLC (Et_2O; R_f (tetraepoxide) 0.43). After completion, the solvent was evaporated: methyl tetraepoxyarachidonate (12 mg, 95%). GC: eight diastereoisomers, three of which are major isomers summing up to 60% of the products. IR (film): 2957, 2931, 2860, 1738, 1456, 1438, 1390, 1250, 1199, 1172, 1011, 847, 827. ^{1}H-NMR: 0.888 (*t*, *J* = 7.0, Me); 1.28–1.91 (*m*, 18 H, CH_2); 2.31–2.47 (*m*, MeOC(O)CH_2); 2.93–3.02 (*m*, H–C(5), H–C(15)); 3.06–3.23 (*m*, 6 H, CH); 3.661 (*s*, MeO). ^{13}C-NMR: 13.94 (Me); 21.83, 21.93 (C(3)); 22.53 (C(19)); 22.12, 22.21 (C(17)); 26.97–27.46 (clustered peaks); 27.78, 27.83, 31.60 (C(18)); 31.63 (C(18)); 33.47 (C(2)); 51.55 (MeO); 53.80–54.25 (clustered peaks); 56.09, 56.39, 56.69, 56.97, 173.55 (C(1)). ESI-MS: 405.2 ([*M* + Na]$^+$). Anal. calc. for $C_{21}H_{34}O_6$ (382.24): C 65.49, H 8.96, O 25.10; found: C 65.47, H 8.95, O 25.08.

4. *Epoxidation of Linoleic Acid in Micelles.* In 100 ml of an aq. medium formed by 0.9M KOH containing 1.12M $NaHCO_3$ (pH 9.5), linoleic acid (29 mg, 0.01 mmol) was suspended. Then 2.7M acetone in H_2O (0.005 mmol, 1.8 ml) was added. The mixture was vortexed until a suspension was obtained. To the vigorously stirred suspension kept at 0°, *Oxone*® (31 mg, 0.005 mmol) was added in portions within 3 h. The mixture was left overnight at r.t. Workup was performed by addition of 1M HCl (pH 4) followed by extraction with $CHCl_3$/EtOH 3:1 (3×). The org. phase was dried ($MgSO_4$) and the solvent evaporated to afford a crude, which was converted to the corresponding methyl ester by standard reaction with diazomethane in Et_2O for the GC and chromatographic analyses. Composition of the crude: starting fatty acid/monoepoxy derivatives 85:15. GC: 1.8:1 ratio of 9,10- and 12,13-monoepoxy derivatives.

5. *Epoxidation of Arachidonic Acid in Micelles. 4-{3-[(2Z,5Z,8Z)-Tetradeca-2,5,8-trienyl]oxiran-2-yl}butanoic Acid* (5,6-EET). Epoxidation of arachidonic acid was carried out by the *in situ* procedure as described in *Exper. 4* for linoleic acid. Two different experiments were carried out varying the amounts of *Oxone*® and acetone for the DMD *in situ* formation, corresponding to 0.8 and 1.9 equiv. compared to the arachidonic acid equiv. In the latter experiment, arachidonic acid (190 mg, 0.63 mmol) was suspended in the standard medium composed by 0.9M KOH containing 1.12M $NaHCO_3$ (pH 9.5). Then 2.7M acetone in H_2O (0.8 mmol, 0.3 ml) was added. The mixture was vortexed until a suspension was obtained. To the vigorously stirred suspension kept at 0°, *Oxone*® was added in portions within 5 h (500 mg, 0.8 mmol). TLC Monitoring (hexane/Et_2O/AcOH 1:3:0.01) was done on aliquots of the mixture treated with 1M HCl (pH 4), extracted with $CHCl_3$/EtOH 3:1 (3×). After 5 h, the mixture was worked up, as described for the aliquot. The solvent was evaporated to give a colorless oil. An aliquot of this crude was converted to the corresponding methyl esters by treatment with diazomethane in Et_2O, and examined by GC. Prod-

uct composition: starting fatty acid (27%), monoepoxy derivatives (46%), and diepoxy derivatives (27%). The composition of the monoepoxy derivatives was deduced from ^{13}C-NMR spectra of the crude, through the relative distribution of the signals of the olefinic C-atoms: 5,6-EET/8,9-EET/11,12-EET/14,15-EET 3 : 3 : 1 : 0.7. Confirmation of this ratio was obtained by isolation after FC (silica gel, TLC eluent): arachidonic acid (R_f 0.70; 51 mg, 0.17 mmol), 14,15-EET (R_f 0.62; 7 mg, 0.021 mmol), 11,12-EET/8,9-EETs (R_f 0.58; 38 mg, 0.12 mmol), and 5,6-EET (R_f 0.50; 29 mg, 0.094 mmol; isolated yield 15%).

The same experiment was repeated with 0.8 equiv. of *Oxone*® after workup and separation as described above, the results reported in *Table 2* were obtained.

6. *Epoxidation of Arachidonic Acid in CCl_4.* DMD (0.8 equiv. of a 0.15M CCl_4 soln., 2.75 ml) was added to arachidonic acid (100 mg, 0.33 mmol; final conc. 0.035M) in CCl_4 (6.6 ml) (TLC monitoring as described in *Exper. 5*). After 10 min, the solvent was evaporated to give a colorless oil. An aliquot of this crude was converted to the corresponding methyl esters by treatment with diazomethane and examined by GC. Final composition: starting material (23%), monoepoxy derivatives (47%), diepoxy derivatives (19%). The composition of the monoepoxy derivatives was deduced from ^{13}C-NMR spectra of the fraction, through the relative distribution of the signals of the olefinic C-atoms: 14,15-EET/11,12-EET/8,9-EET/5,6-EET 4.5 : 3.3 : 1.3 : 1.

The experiment was repeated with 1.9 equiv. of DMD (0.627 mmol, 4.2 ml of a 0.15M CCl_4 soln.) and arachidonic acid (100 mg, 0.33 mmol; final conc. 0.030M) in CCl_4 (6.6 ml). The analysis was performed as described above: monoepoxy derivatives (23%), diepoxy derivatives (44%), and triepoxy derivatives (24). Only 2% of starting material was recovered. ^{13}C-NMR analysis: 14,15-EET/11,12-EET/8,9-EET 3 : 3 : 1 (*Table 2*).

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